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# Age-related decrements in the muscarinic enhancement of $K^+$-evoked release of endogenous striatal dopamine: an indicator of altered cholinergic–dopaminergic reciprocal inhibitory control in senescence

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Previous experiments have indicated that the release of striatal dopamine (DA) is controlled by inhibitory DA autoreceptors which are mediated by inhibitory cholinergic heteroreceptors (HTRs). Activation of the HTRs by muscarinic or nicotinic agonists potentiates the $K^+$-evoked release of DA from the striatum. Present experiments were carried out to determine if this relationship is altered as a function of aging. Cross-cut striatal tissue slices obtained from 3 age-groups (6, 12–18 and 24 months) Wistar rats were superfused with a modified Krebs–Ringer basal release medium containing 2.5 mM KCl. After a 30-min equilibration period, a 5-min baseline fraction was collected from each chamber. The medium was then switched to one containing 30 mM KCl, and depending upon the experiment, 1 of 4 concentrations of a particular muscarinic (oxotremorine, pilocarpine, carbachol or bethanecol) or nicotinic (nicotine) agonist. In some experiments DA autoreceptor function was assessed directly with haloperidol. Six 5-min fractions were taken during depolarization. DA release was assessed using high performance liquid chromatography coupled to electrochemical detection. Results indicated that the efficacy of the muscarinic agonists was reduced in an age-dependent manner with the oldest age groups showing the smallest enhancement. The age at which the decline was seen was dependent on the muscarinic agonist that was applied. Deficits were seen as early as 12 months when full agonists (e.g. carbachol) were applied, but did not appear until 18 months when partial agonists (e.g. oxotremorine) were applied. These age-related alterations were not seen when haloperidol or nicotine were used to enhance the $K^+$-evoked release of DA. Results are discussed in terms of specific deficits in the muscarinic control of striatal DA autoreceptors in senescence. These decrements occur as result of age-related alterations in the conformational/orientational state of the muscarinic receptors regulating striatal DA release.

## INTRODUCTION

Evidence derived from numerous sources indicates that there are very significant changes occurring within both the striatal dopamine (DA) and acetylcholine (ACh) systems during aging. The exact specification of these alterations is dependent upon both the species examined and the conditions of the experiment, but indications are that some of the most striking changes take place in the various populations of receptors from these systems. In the case of the striatal DA system, the receptors were originally divided into two subtypes ($D_1$ and $D_2$ ref. 17), and subsequent findings indicated that each receptor subtype showed a different pattern of change with age. It appeared that while the $D_2$-receptor showed remarkably consistent age-related declines in concentration, the changes occurring in the concentration of striatal $D_1$-receptors was much more variable. Initial assessments of $D_2$-receptor loss with age indicated a 35% decrease in binding with no loss of binding affinity in the aged rat. Subsequent observations have indicated that the degree of $D_2$-receptor loss is dependent upon the assay conditions, the strain or species of animal tested, and the particular dopamine-sensitive ligand that is utilized, but this decline generally ranges from about 36 to 66%[11,15,31,32].

If age-related changes in the $B_{max}$ or $K_d$ of the stria-

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tal $D_1$-receptor are considered, the results from at least one laboratory show parallel losses between $D_1$-receptor concentration and DA-stimulated adenylate cyclase activity that are the greatest between 3 and 12 months of age in the rat[11]. However, findings from laboratories using other strains of rats (e.g. Sprague–Dawley[24]) or mice (e.g. C57BL6 ref. 24) indicate no change in the concentration of striatal $D_1$-receptors with age. Examination of postmortem human material indicates an actual increase in $D_1$-receptors with age[22]. Since the changes in the concentration of striatal $D_2$-receptors seem to follow a progressive decline throughout the life-span of the organism, while changes in the concentration of striatal $D_1$-receptors do not, these results suggest that there may be increases in the striatal $D_1/D_2$-receptor ratio in senescence, a finding which has been confirmed in humans[22] and rats[11].

Assessments of age-related differences in striatal muscarinic cholinergic receptor binding indicate that there are decreases in the number of these binding sites in the striatum as a function of age. Declines in the concentration of striatal muscarinic receptors of about 25–29% in the rodent have been reported in studies using the ligand, [$^3$H](–)-quinuclidinyl benzilate ([$^3$H]QNB[23]). These declines were seen primarily in the medial and caudal portions of the striatum[34], a pattern that approximates that seen with respect to loss of $D_2$-receptors.

One question that arises from these findings is that since there appears to be a great deal of interactive modulation that takes place between the striatal DA and ACh systems, how do age-related declines in their concentrations alter these respective interactions? This question has not received much systematic study, but there are indications that there may be age-related declines in the normal reciprocal inhibitory control (RIC) that is exerted between these two systems. Recent studies have shown that while striatal DA-stimulated adenylate cyclase activity is inhibited by muscarinic agonists such as oxotremorine or carbachol in striatal broken cell preparations prepared from young rats (6 months), these agents were ineffective in inhibiting this activity in senescent rats (24 months)[4].

Additional assessments carried out to examine DA modulation of [$^3$H]ACh release from striatal slices from mature, middle-aged and senescent rats indicated that the DA agonist, apomorphine, was effective in inhibiting $K^+$-evoked release of [$^3$H]ACh from superfused striatal slices obtained from mature and middle-aged rats but not from those prepared from old rats[35].

These findings, when considered in light of those discussed above indicate that as receptors from both of these transmitter systems are lost with age, alterations in one may profoundly affect the functioning of the other, and RIC that is normally exerted between these two systems is significantly diminished. The present experiments were directed toward further examination of age-related alterations in cholinergic regulation of striatal DA autoreceptors. It is now widely accepted that striatal DA release is under the control of a group of inhibitory DA autoreceptors[1–7,12,13,16,18,25,26,33,40]. If these autoreceptors are stimulated via DA agonists or inhibited with DA antagonists, $K^+$-evoked release of DA will be respectively inhibited or enhanced. Normally, this control is in turn mediated through inhibitory muscarinic and/or nicotinic heteroreceptors[19,27,28,30,41] presumably located on the same terminals as the autoreceptors[10]. Either muscarinic (e.g. oxotremorine, carbachol, bethanecol) or nicotinic (e.g. nicotine) agonists can activate the heteroreceptors, which inhibit the DA autoreceptor and potentiate the $K^+$-evoked release of DA from the striatum[27,28]. The actions of the muscarinic agonists on DA release can be attenuated by both atropine as well as the more selective $M_1$-muscarinic antagonist pirenzepine[26].

Thus, enhancement of DA release was assessed in superfused striatal slices obtained from young, middle-aged, and old rats following application of muscarinic or nicotinic agonists and muscarinic antagonists. Additionally, in order to examine any age-related alterations in DA autoreceptor function directly, the potentiation of $K^+$-evoked DA release by haloperidol was also determined.

## MATERIALS AND METHODS

### *Procedure*

Striatal tissue from mature (6 months), middle-aged (12 and 18 months), and aged (24 months) Wistar rats was obtained (following sacrifice by decapitation) from the Gerontology Research Center, Baltimore, MD (in some experiments striata from only 6-

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and 24-month rats were utilized; see below). Crosscut striatal slices (0.3 μm) were prepared using a McIlwain tissue chopper. Slices from equal numbers of animals were pooled for each age group and placed in small glass vials containing a modified Krebs–Ringer basal release medium that had been bubbled for 30 min with 95% $O_2$/5% $CO_2$ and contained (in mM) $NaHCO_3$ 21, glucose 3.4, $NaH_2PO_4$ 1.3, EGTA 1, $MgCl_2$ 0.93, NaCl 127 and KCl 2.5 (pH 7.4). The slices were washed twice in this medium and 150 μl aliquots (approximately 1.1–1.4 mg tissue/chamber) were placed in a superfusion apparatus containing 16 parallel chambers. Typically, pooled tissue from 2 animals was used to fill 4 of the parallel chambers. The tissue and buffer media were maintained at 37 °C throughout the course of the experiment. Following placement into the superfusion chambers, the tissue was allowed to equilibrate for 30 min. It was continuously perfused with the oxygenated basal release medium at the rate of 124 μl/min. After the equilibration period, a 5-min baseline fraction was collected and the medium was then switched to one containing (in mM) KCl 30, $CaCl_2 \cdot 2H_2O$ 1.26 (in place of EGTA) and NaCl 57, as well as the other components described above (pH 7.4). Following the switch, 5-min fractions continued to be collected for 30 min. To this oxygenated release medium was added 1 of 4 concentrations of the agent under study ([(0 μM, 100 μM, 500 μM, and 1 mM of oxotremorine, carbachol, pilocarpine, bethanecol, or nicotine); (haloperidol, 0 μM, 10 μM, 100 μM, 500 μM)]. In addition, the specificity of any muscarinic agonist enhancement of KCl-induced release of DA was examined following application of 500 μM oxotremorine in the presence or absence of graded concentrations of atropine (100 μM, 500 μM, 1000 μM) or the selective $M_1$ antagonist pirenzepine (500 μM, 1000 μM, 2000 μM). Although only one agonist or antagonist was studied in a particular experiment, all concentrations were represented in that experiment. The fractions were collected into tubes on ice containing 0.3 ml of cold 0.1 N perchloric acid, 0.05% sodium metabisulfite, and 0.1% EDTA. These samples were then stored at −80 °C until assessment of DA release via high performance liquid chromatography (HPLC) coupled to electrochemical detection.

The HPLC consisted of a varian model 5000 ternary chromatograph, a varian model 8055 autosampler, and a valco air actuated injector with a 50 μl loop. The effluent was monitored with a bioanalytical systems LC-4B amperometric detector using a glassy carbon electrode. The detector potential was set at 0.72 V as a $Ag/AgCl_2$ reference electrode with a sensitivity of 10 nA/V. The mobile phase consisted of a filtered, degassed 100 mM $KH_2PO_4$ buffer containing 3 mM 1-heptanesulfonic acid, 100 μM EDTA, and 8% (V/V) acetonitrile (pH 3.6). The components were eluted off a Waters 10 μm particle, μBondapakC$^{18}$ reverse-phase column (30 × 0.39 cm; flow rate = 1 ml/min) maintained at 30 °C. Results were calculated relative to

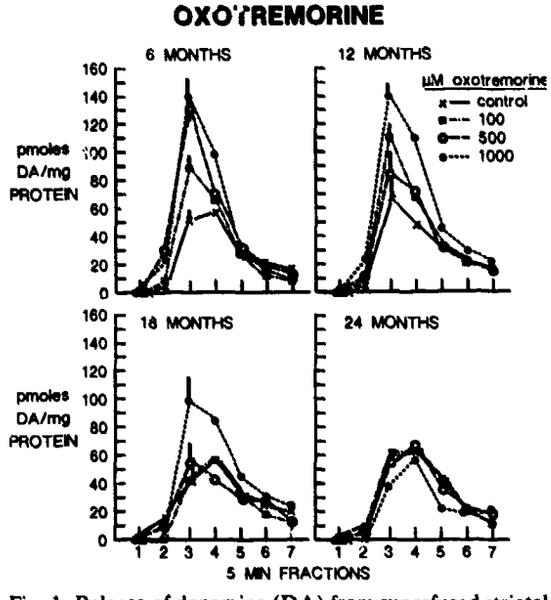


Fig. 1. Release of dopamine (DA) from superfused striatal tissue slices following stimulation by 30 mM KCl and 1 of 4 concentrations of oxotremorine. Striata were obtained from Wistar rats of 4 age groups. The slices were placed in a superfusion apparatus containing 16 chambers. Pooled tissue from two animals was used to fill 4 chambers. The slices were allowed to equilibrate for 30 min and were continuously perfused with oxygenated basal release medium (see Methods) at the rate of 124 μl/min. After the equilibration period a 5 min baseline release fraction was collected. The medium was then switched to one containing the 30 mM KCl and one of the compounds under study. Six additional 5-min fractions were then taken. Dopamine release was analyzed by high performance liquid chromatography coupled to electrochemical detection. Note that the peak release of DA was not seen until the second fraction after the switch (3 on the *x*-axis). This delay is the result of the time required in getting the high KCl medium from the reservoir to the tissue and then to the collection vial. The size (PE50), and length (112 cm) of the tubing as well as the flow rate dictated this delay to be about 5–7 min after the switch.

known standards that were analyzed on the HPLC at the same time as the samples. Samples were run in an autosampler and standards were placed at the beginning, middle and end of the sequence. Concentrations of the components were determined from areas under each peak when compared to the conversion factors derived from the standards. The lower limit of the sensitivity of this assay was 250 fmol. In order to ensure that DA was being assessed retention times were measured using DA standards. Additionally, pilot experiments were carried out to determine if DA 'spikes' added to samples enhanced the DA peaks on the chromatograms. Data were expressed as pm/mg protein as analyzed by the Lowry[21] procedure.

*Data analysis*

In experiments where 4 age groups were represented (i.e. those which examined enhanced release of striatal dopamine induced by 30 mM KCl co-applied with oxotremorine, carbachol, pilocarpine, or bethanecol) data were analyzed using 4 (age) by 4 (drug concentrations) by 7 (fractions) repeated measures (on the last factor) analyses of variance. In experiments where nicotine, haloperidol, and atropine were examined, striata from the two groups of middle aged animals were not studied. Therefore, 2 (age) by 4 (drug concentrations) by 7 (fractions) analyses of variance were carried out. Further comparisons among the various groups were analyzed by post hoc *t*-tests[42].

## RESULTS

*Muscarinic agonists and the enhancement of $K^+$-evoked release of dopamine*

Generally, as can be seen from Figs. 1–4, the release of DA in striatal slices obtained from all of the age groups was increased over basal release when a depolarizing concentration of KCl (30 mM) was introduced into the superfusion medium. These figures also show that in the striata obtained from the 6-month animals there was a concentration-dependent enhancement of the $K^+$-evoked release of DA by oxotremorine (drug concentration by fraction — $F_{18,546} = 9.34$, $P < 0.0001$, Fig. 1; pilocarpine $F_{18,366} = 8.14$, $P < 0.0001$, Fig. 2; carbachol, $F_{18,234} = 1.97$, $P < 0.01$, Fig. 3; and bethanecol $F_{18,402} = 3.27$, $P < 0.01$, Fig. 4). However, Figs. 1–4 also show that there were significant declines in the ability of these agonists to enhance $K^+$-evoked release of DA as a function of age (drug concentration by fraction by age — oxotremorine, $F_{54,546} = 1.72$, $P < 0.001$; pilocarpine, $F_{54,366} = 1.62$, $P < 0.001$; bethanecol, $F_{54,402} = 3.83$, $P < 0.0001$; carbachol, $F_{54,234} = 1.71$, $P < 0.001$).

The pattern of these age-related declines was highly dependent upon the particular agonist that was examined. Enhancement of $K^+$-evoked release of DA was generally higher in striata obtained from the 6-month groups than those from the 18- and 24-month groups when either oxotremorine or pilocarpine (from 100 $\mu$M to 1000 $\mu$M) was co-applied with 30 mM KCl (post hoc *t*-analyses, all comparisons of either 6-month groups with those of 18- or 24-months, $P < 0.05$). Similarly, significant differences in enhancement of $K^+$-evoked release by oxotremorine were also observed between the 12-month groups and the older age groups (e.g. oxotremorine, 100 $\mu$M, 12 vs 18 months — $t = 5.62$, $P < 0.001$; 500 $\mu$M, $t = 3.07$, $P < 0.01$; 1000 $\mu$M, $t = 4.35$, $P < 0.05$, df = 546; pilocarpine 1000 $\mu$M, $t = 1.96$, $P < 0.05$, df

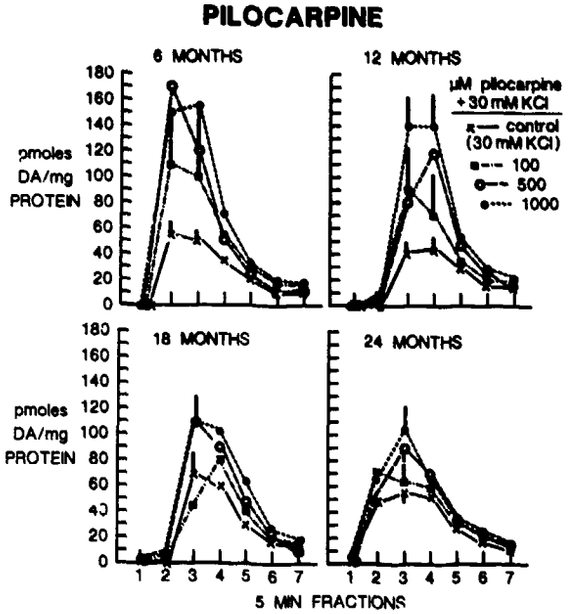


Fig. 2. Effects of pilocarpine on the $K^+$-evoked release of dopamine from superfused striatal tissue slices obtained from different aged rats (6, 12, 18, and 24 months).

= 366). There was also some evidence of continued further decline in this aspect of RIC between 18 and 24 months, since subsequent post hoc $t$-analyses indicated significant differences between these two groups at the higher concentrations of both pilocarpine and oxotremorine (e.g. oxotremorine, 1000 $\mu$M, $t = 4.35$, $P < 0.001$, df = 546; pilocarpine, 500 $\mu$M, $t = 3.10$, $P < 0.02$, df = 366).

In contrast to the findings seen with respect to oxotremorine and pilocarpine, when carbachol was used to enhance $K^+$-evoked release of DA there were indications that significant decrements in this enhancement appeared as early as 12 months of age (Fig. 3). Subsequent analyses indicated that there was less enhancement of $K^+$-evoked release of DA in the striata from the 12-month group than those from the 6-month group for all concentrations of carbachol (i.e. 100 $\mu$M, $t = 2.11$, $P < 0.02$; 500 $\mu$M, $t = 5.15$, $P < 0.001$; 1000 $\mu$M, $t = 5.55$, $P < 0.001$, df = 254). In fact, the only enhancement of $K^+$-evoked release of DA that was observed in any of the striatal slices was seen in the 6-month group. Carbachol did not enhance DA release from the striata of any of the other groups above that seen with the application of KCl alone (all within-age-group $t$-tests between 0 $\mu$M and other concentrations of carbachol in the 12-, 18-, and 24-month age-groups $P > 0.05$).

When bethanecol was used to enhance the $K^+$-evoked release of DA from striatal slices it can be seen from Fig. 4 that the results were similar to those seen with respect to carbachol in that the age-related deficits in the efficacy of bethanecol appeared in striata from the 12-month group (6 vs 12 months — 100 $\mu$M, $t = 3.05$, $P < 0.02$; 500 $\mu$M, $t = 4.72$, $P < 0.001$; 1000 $\mu$M, $t = 5.96$, $P < 0.001$, df = 400) and continued into the 18- and 24-month groups (e.g. 6 vs 18 months — 500 $\mu$M, $t = 4.55$, $P < 0.001$; 6 vs 24 months — 500 $\mu$M, $t = 5.29$, $P < 0.001$, df = 400). Enhancement of the release DA by the various concentrations of bethanecol in the striata from the 12 month group did not differ from that seen in the striata from the 18 month group. Response differences between the striata from the 12- and 24-month groups were only seen when 500 $\mu$M bethanecol was co-applied with 30 mM KCl ($t = 2.55$, $P < 0.01$, df = 400). These findings indicate that the age-related pattern of declines in responses tended to be similar between oxotremorine and pilocarpine wherein they were seen to begin in the 18- to 24-month groups,

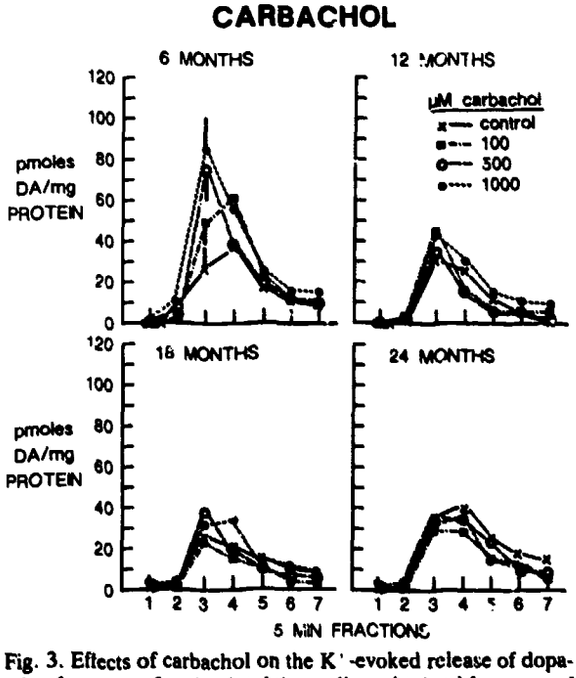


Fig. 3. Effects of carbachol on the $K^+$-evoked release of dopamine from superfused striatal tissue slices obtained from rats of 4 different ages (6 through 24 months).

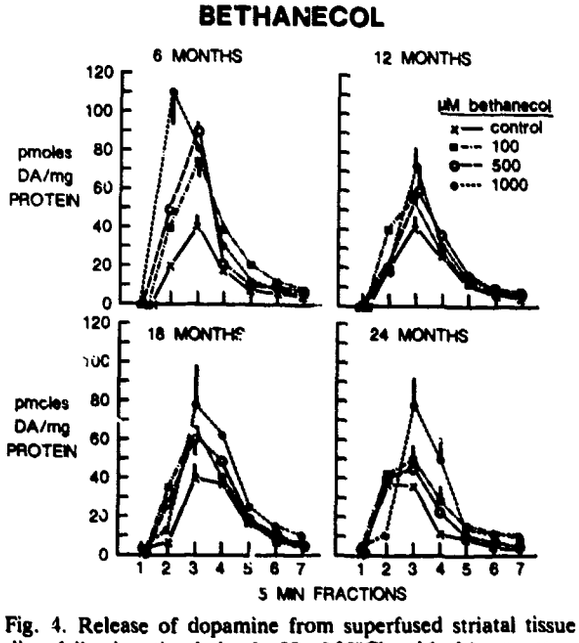


Fig. 4. Release of dopamine from superfused striatal tissue slices following stimulation by 30 mM KCl and 1 of 4 concentrations of bethanecol.

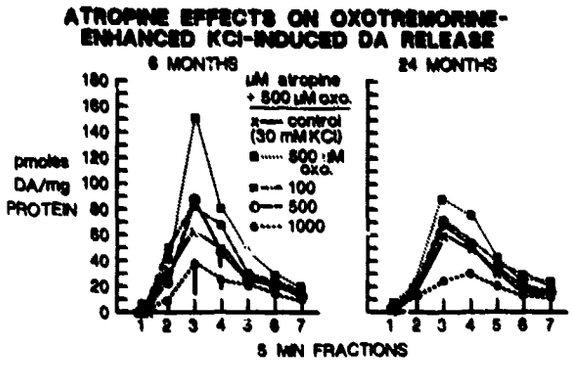


Fig. 5. Atropine antagonism of oxotremorine-enhanced, $K^+$-evoked release of dopamine from superfused striatal tissue slices (6- and 24-month animals).

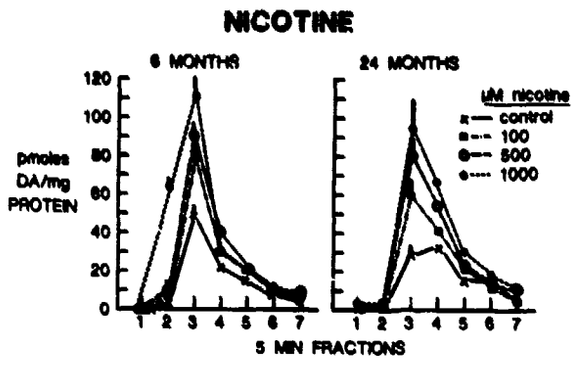


Fig. 7. Nicotine enhancement of 30 mM KCl-induced release of dopamine from superfused striatal tissue slices (6- and 24-month animals).

while the findings with respect to carbachol and bethanecol indicated that the deficits appeared as early as 12 months of age.

Importantly, as Fig. 5 indicates, there was a concentration-dependent antagonistic effect of atropine on oxotremorine-enhanced $K^+$-induced release of striatal DA (concentration by fraction — $F_{24,126} = 4.03$, $P < 0.0001$). Similar findings were seen with respect to pirenzepine (concentration by fraction — $F_{24,120} = 4.44$, $P < 0.0001$) (Data not shown).

*Nicotine enhancement of $K^+$-evoked release of DA*

Fig. 6 shows that unlike the muscarinic agonists, nicotine was much more effective in enhancing KCl-induced release of DA in the striata from old animals. While analysis of variance indicated that age-related differences were still present between the two age groups (age by fraction by concentration — $F_{18,384} = 1.64$, $P < 0.05$). Further analyses showed that these differences were primarily the result of age-related differences in peak enhancement at the 100 $\mu$M concentration of nicotine ($t_{384} = 3.00$, $P < 0.02$). No significant age-differences were seen at higher concentrations.

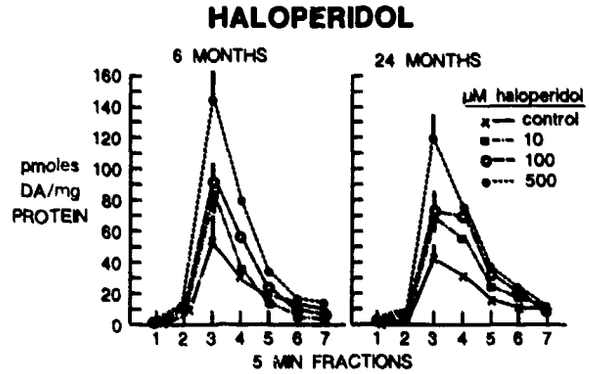


Fig. 6. Haloperidol enhancement of 30 mM KCl-induced release of dopamine from superfused striatal tissue slices obtained from rats 6 and 24 months of age.

*Haloperidol-induced striatal DA antagonism*

Direct DA autoreceptor inhibition with varying concentrations of haloperidol revealed only small age-related differences in the enhancement of KCl-induced release of DA (Fig. 7). While there was some overall lowering of striatal responsivity in the 24-month group (age by fraction by concentration — $F_{18,312} = 1.83$, $P < 0.05$), subsequent analyses indicated that each concentration of haloperidol was effective in enhancing the $K^+$-evoked release of striatal DA (24-month within-age comparisons — 0 $\mu$M vs 100 $\mu$M, $t = 6.75$, $P < 0.001$; 100 $\mu$M vs 500 $\mu$M, $t = 1.76$, $P < 0.05$).

## DISCUSSION

As stated in the Introduction, there are rather profound alterations in the various populations of striatal DA receptors, especially $D_2$-receptors, that take place as a function of age. There are also indications from at least one recent study that the striatal DA autoreceptor is the $D_2$-receptor[6]. Thus, it is interesting to note in this regard that, at least with respect to some of the independent variables assessed, the remaining $D_2$-autoreceptors appear to show only mini-

mal age-related changes in function. Age-related differences in the $K^+$-evoked release of DA from the striatal tissue slices were small in these experiments. These findings support those of earlier experiments[36], but appear to be somewhat different from those in which in vivo electrochemical analyses of striatal DA release was utilized to examine possible age-related differences in this parameter. A recent study by Rose et al.[29], in which this latter technique was employed, has indicated that two groups of 24-month-old animals showed no differences in $K^+$-evoked release of striatal DA from that exhibited by 6-month animals. However, the third group of 24-month animals did show significantly lower $K^+$-evoked release of DA when compared to the 6-month group. It is unclear why these within age-group differences exist since the experimental conditions were the same in all of the groups. The authors suggest there may have been slight differences in the rostrocaudal placement of the electrodes in these animals, since, as pointed out above, there are intraregional striatal variations in other indices of synaptic function. Therefore, some of the electrodes could have been placed into areas of the striatum that are relatively unaffected by aging.

Further evidence that DA autoreceptor function remains relatively unchanged during aging is indicated from the findings showing that there were only small between-group differences seen when the $K^+$-evoked release of DA was enhanced with haloperidol. For example, in the presence of 30 mM KCl and 500 $\mu$M haloperidol the DA release was enhanced about 37% in both the 6- and the 24-month groups.

One other source of evidence which indicates that there are no significant age-related declines in DA autoreceptor function can be found from the present results that indicate that there were only small differences in nicotine-enhanced $K^+$-evoked release of DA in the striata obtained from the 6- and 24-month groups. Also important in this regard is that reciprocal inhibitory control between nicotinic heteroreceptors and DA autoreceptors is not lost with age. However, if RIC between muscarinic heteroreceptors is considered, there are strong indications of profound age-related changes. The results showed that there were selective and significant decreases in enhancement of KCl-induced release of DA from striatal slices by muscarinic agonists as function of age. These decreases were highly dependent on the particular agonist that was used to enhance the release. Oxotremorine and pilocarpine were extremely effective in enhancing DA release and the release was generally greater when these partial agonists were applied than when a full agonist such as carbachol was placed in the medium. Age-related decrements in muscarinic control of the DA autoreceptor-mediated release of DA appeared as early as 12 months of age when carbachol was applied to the slices.

Previous studies (e.g. refs. 8, 9) have indicated that, based upon their ability to stimulate phosphoinositide turnover, these muscarinic agonists can be divided into two classes. Class A agonists, such as carbachol, maximally stimulate phosphatidate and inositol lipids from hippocampus and cortex, while partial agonists such as oxotremorine and pilocarpine only poorly stimulate phosphoinositide turnover. It is believed that class A agonists such as carbachol can alter the microenvironment of the muscarinic receptor (mAChR) allowing a change in the conformation/orientation of the receptor to a desensitized state, wherein the receptor is uncoupled from the effector mechanism[20]. During aging, recent studies have suggested that, beginning with middle age, conformation/orientation changes take place in mAChR such that more mAChR are in a desensitized, decoupled state[20]. If an agonist is applied which further desensitizes the mAChR, deficits in responding should be seen earlier in the lifespan than if an agent is utilized which does not induce decoupling of the receptor from the effector. Thus, age deficits are seen earlier with carbachol than with oxotremorine or pilocarpine.

Bethanecol, which was previously classed as a B (partial) agonist in hippocampus and cortex, behaves very much like an A agonist in the striatum and reductions in its efficacy are seen as early as 12 months of age. Preliminary results (not presented here) have indicated that the threshold concentration of bethanecol was 10 $\mu$m in striata obtained from the 6-month group and 100 $\mu$M in all other groups.

In summary then, the results of this and previous experiments indicate that although the age-dependent decreases in the concentrations of particular striatal DA subtypes do not appear to appreciably affect the functioning of those that remain, this loss combined with the decrease in concentration of stria-

tal mAChR and the increased heterogeneity of the mAChR can have a profound effet on the RIC that is normally exerted between these two systems. Since one can assume that proper striatal functioning depends upon the precise interplay between the ACh and DA systems, any age-related changes in their relationship could subsequently involve a myriad of other neurotransmitters and neuromodulators and be translated ultimately into motor behavioral deficits (i.e. decrements in balance, coordination, and sensory motor integration[14,15,37,39]).

At present it is unclear what is responsible for the loss of striatal dopaminergic and cholinergic receptors in senescence. The relationship between receptor loss and neuronal loss is still controversial and investigations are being carried out in our laboratory as well as others. However, even if we were to suggest that the former is the result of the latter, this would not explain the selectivity of the age-related functional deficit in the mAChR, even though both DA and mAChR are lost with age. Moreover, it does not explain the mechanism of this functional loss.

One mechanism that is being explored to investigate this loss is that there may be accumulated membrane damage by free radicals. Some preliminary data from our laboratory in support of this hypothesis have indicated that young animals irradiated with either high energy electrons or $^{56}Fe$ radiation (which are known to generate free radicals) also show reduced muscarinic enhancement of $K^+$-evoked release of DA. These effects are coupled with motor behavioral deficits that resemble those seen in the senescent animal. Thus, some behavioral deficits seen with respect to aging and ionizing radiation may share a common mechanism, i.e., that of free radical-induced neuronal damage.

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